Colony PCR and Overnight Cultures of LT10 Promoter Transformation

Made with Benchling

Project: Awesome Possum

Authors: Cara Jones

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Purpose:

Run PCR and overnight cultures of picked colonies from LT10 Promoter transformation plates.

Materials:

- Phusion HF Buffer
- 10 mM DNTP
- LTNF Forward Primer
- LTNF Reverse Primer
- Phusion DNA Polymerase
- dH_20
- LB Broth
- Chloramphenicol
- Culture tubes
- PCR tubes
- Thermal Cycler
- Shaker/incubator
- Micropipette
- Micropipette Tips
- Overnight LT10 Promoter Transformed Cell Colony Plates

Protocol:

A Master mix of PCR reagents (see Table 1 for values) was created and 49.0 µL of mix was aliquoted into 10 PCR tubes. Then 10.0 mL of LB Broth was dispensed into 10 culture tubes with 10.0 µL of chloramphenicol each. After that, 10 colonies on the cell colony plate were identified and labeled. Next, each colony was sampled with a micropipette tip, swirled into a PCR tube, and then dropped into a culture tube. Then the culture tubes were placed into a shaker/incubator overnight at 37.0˚C 250 RPM. Next, the PCR tubes were placed into a thermal cycler at the following settings:

The thermal cycler was set as following:

1. 95.0˚C 3.0 minutes
2. 95.0˚C 30.0 seconds
3. 62.0˚C 30.0 seconds
4. 72.0˚C 1.0 minutes
5. Repeat steps 2-4 for 12 cycles
6. 72.0˚C 5.0 minutes
7. 4.0˚C ∞

Table 1: PCR Mastermix Reagent Volumes

	A	B	C	D	E	F
1	5x Phusion HF Buffer	10 mM DNTP	LTNF Forward Primer	LTNF Reverse Primer	Phusion DNA Polymerase	dH20
2	100.0 µL	10.0 µL	25.0 µL	25.0 µL	5.0 µL	315.0 µL